In the US, congenital heart defects (CHD) are among the most common birth defects, occurring in approximately 1/100 live births. The majority of CHDs involve abnormal valvulo-septal development, however, the molecular mechanisms underlying valve development remain relatively unknown. The T-box family of transcription factors is known to be involved in several aspects of heart development including cardiac lineage determination and chamber specification. Recent studies suggest that Tbx20 is a likely candidate for regulating valve development. In murine embryos, tbx20 is expressed in the myocardium where it is required for proliferation. Tbx20 is also expressed in the endocardial cushions (EC) of the atrioventricular canal and remodeling valves, however, its function is unknown. Upstream regulators and downstream targets of Tbx20 were examined in avian EC cells in order to elucidate the function of Tbx20 in developing valves. Previous studies have shown that Bmp2 is necessary for EC formation. We show that, in EC cells, tbx20 is induced by the Bmp2 pathway. In addition, Tbx20 gain and loss of function studies demonstrate that Tbx20 increases the expression of extracellular matrix genes including aggrecan and versican and decreases the expression of matrix metalloproteinases including mmp9 and mmp13. Additionally, Tbx20 promotes proliferation in EC cells. Taken together, these data support a role for Tbx20 in proliferation and extracellular matrix remodeling during valve development.

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Snail family genes are required for left–right asymmetry determination but not neural crest formation in mice

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Members of the *Snail* gene family regulate epithelial–mesenchymal transitions (EMT) by repressing the transcription of components of cell adhesion complexes. EMT occurs several times during development, including gastrulation and delamination of neural crest cells from the dorsal neural tube. We reported previously that *Snail (Snai1)* null mouse embryos displayed gastrulation defects, which die by 7–8 days of gestation, precluding examination of the role of *Snai1* in later developmental events. Using a conditional allele and the Meox2-cre line, we are able to rescue the *Snai1* null phenotype through 9.5 days of gestation. Here, we show that, contrary to observations in frog and avian embryos, *Snai1* and *Slug (Snai2)* are not required for formation and delamination of the neural crest in mice. In both $Snai1^{flox/-}$; Meox2-Cre *(Snai1-cko)* and *Snai1-cko*;$Slug^{-/-}$ compound mutant embryos, neural crest cells form, delaminate and appear to migrate properly into the branchial arches. However, in addition to gastrulation defects, the *Snai1-cko* embryos exhibit multiple laterality defects, including randomization of the direction of heart looping and embryonic turning. The left determinant genes *Nodal*, *Lefty2* and *Pitx2* display bilateral expression patterns that are particularly prominent in the posterior region, overlapping the normal *Snai1* expression domain. These changes are independent of gross structural defects at the midline and in the node. Our findings suggest that *Snai1* is not required for delamination of the neural crest in mice but plays a critical role in the determination of left–right asymmetry.

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Foxd3 is required for neural crest development

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The neural crest (NC) cells disperse from the dorsal surface of the neural tube and migrate extensively through the embryo, giving rise to a wide variety of differentiated cell types. Foxd3, a member of winged helix transcription factor family, is expressed early in the preimplantation and gastrulating embryo, later in the premigratory and migrating neural crest and some differentiated NC derivatives. Previous studies showed that Foxd3 is sufficient to direct neural cells towards the NC lineage and is required for maintenance of two disparate stem cell types from the early mouse embryo: embryonic stem cells and trophoblast stem cells. The null mutant embryos die around 6.5 dpc, before the NC is specified. Here, we conditionally inactivated the *Foxd3* gene specifically in the NC using the Cre/LoxP recombinase system. $Foxd3^{flox/-}$;*Wnt1-cre* embryos die perinatally with profound deficiencies in cranial, trunk, vagal and sacral neural crest, including severe craniofacial cleft and missing bones of the head, hypoplastic cranial nerves and pharyngeal arches, defects of peripheral nervous system and complete absence of enteric nervous system. However, the heart and its outflow tract appear grossly normal. $Foxd3^{flox/-}$;*Wnt1-cre* embryos have apoptotic cells located at the dorsalmost region of the neural tube where wild type embryos do not, and cell proliferation is unchanged. Our results establish a requirement for Foxd3 in NC development. Experiments are in progress to understand the interaction of Foxd3 with signal transduction networks and transcription factors controlling migration and differentiation of NC in vivo and maintenance of NC stem cells in vitro.

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Early specification of neural crest and the role of Pax7 on its development

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Neural crest cells are a dynamic migratory stem cell population that differentiates into a plethora of derivatives,

including cells of the peripheral nervous system, endocrine cells, melanocytes, and craniofacial bone and cartilage. Our current understanding of neural crest formation, specifically of cranial crest, is very limited despite their relevance in frequent birth defects (cleft pallet). Neural crest cells are thought to originate at the border between neural and non-neural ectoderm. We have initiated studies aimed at understanding the early induction of neural crest cells, including the cranial crest. To this end, we performed an in vitro specification assay under non-inducing conditions in early chick embryos. We have found that neural crest induction is underway during gastrula stages 3 to 4 in a restricted medial epiblast region, which is competent to generate neural crest cells in the absence of additional tissues or signals. This medial epiblast will soon express pax7 (stage 4+ to 5), and the expression of Pax7 will label the neural folds and migrating neural crest cells later on. By preventing Pax7 translation with morpholinos, we unveil its early requirement for neural crest formation in vivo. These data suggest that neural crest specification initiates much earlier than previously assumed and that Pax7 plays a critical role in the early steps towards crest.

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An NF-κB, Slug and Wnt network in *Xenopus*

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During neural crest formation and cancer cell metastasis, cells undergo a dramatic epithelial to mesenchymal transition (EMT) combined with the suppression of anoikis, a form of apoptosis that occurs when epithelial cells lose contact with their substrate. The related zinc finger transcription factors Slug and Snail have been implicated in the regulation of both processes. In the course of studies to separate these two functions during neural crest formation in *Xenopus*, we found that RelA, which encodes an NF-κB subunit protein, is positively regulated by Slug. RelA directly up-regulates expression of Slug, Snail, the neural crest marker Sox9, as well as the anti-apoptotic BclxL gene and inhibits accumulation of RNA encoding the pro-apoptotic protein p53. Both RelA and BclxL rescue the effects of blocking Slug expression on neural crest formation, and a dominant-negative form of RelA disrupts neural crest formation. Based on the use of dominant negative forms of IκB and acetyl-11-keto-β-boswellic acid, an inhibitor of IκB kinase activity, it appears that BclxL acts through an NF-κB/IκB-dependent process to regulate both RelA and Slug expression. To complete the regulatory circuit, we find that *Drosophila* WntD and *Xenopus* Wnt8 inhibit RelA RNA accumulation. Taken together, these observations indicate that a circuit analogous to the *Drosophila* Dorsal (NF-κB)/Snail (Slug) circuit is active in vertebrates and that NF-κB activity is required for neural crest formation in *Xenopus*. Work supported by NIH grant GM54001 and the American Heart Association.

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C. elegans Sp1 factor LEX-4 functions with the Wnt pathway

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The Sp1 transcription factors play an important role in the development of several organisms. There is evidence that the Sp1 members function in regulating Wnt pathway in both invertebrates and vertebrates. This study reports the cloning of the *C. elegans* Sp1 gene, *lex-4*, and elucidates its potential role in modulating Wnt regulated processes.

We isolated *lex-4(gu85)* in a genetic screen performed to identify partners that function with EGL-38, a Pax factor, in regulating the expression of a target gene *lin-48*. SNP mapping placed the *lex-4(gu85)* mutation on LGI, and sequencing of the genomic DNA revealed that it is a mutant allele of the Sp1 homolog Y40B1A.4. We find that *gu85* is a missense mutation that affects the DNA binding domain and abolishes the DNA binding ability of the protein. In addition, *lex-4(gu85)* mutant animals show a wide range of developmental defects that are similar to these seen in Wnt pathway mutants. Specifically, mutant hermaphrodites exhibit the Biv phenotype similar to *lin-17(frizzled)* and *lin-18(RTK)* mutants. We have used vulval cell markers that suggest that the Biv phenotype results from reversal of the P7.p cell lineage characteristic of the Wnt pathway mutants. We are currently working on dissecting the mechanism of action of the *lex-4* gene in the development of several organs that are responsive to the Wnt pathway and are attempting to determine how this gene functions with the Wnt pathway to influence development.

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Activation of *Goosecoid* transcription by Siamois and Twin

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In early *Xenopus laevis* development, the Spemann organizer regulates the patterning of the mesoderm at the marginal zone and is essential in the establishment of a complex and organized body plan. The transcription factors *siamois* (Sia) and *twin* (Twn) are expressed in the dorsal vegetal blastomeres in response to stabilized β-catenin and are thought to be key regulators of organizer gene expression. Sia and Twn are among the earliest organizer genes expressed following